

Preparation and characterization of highly pure fractions of outer membrane, cytoplasmic and intracytoplasmic membranes from *Ectothiorhodospira mobilis*

TONI DITANDY and JOHANNES F. IMHOFF*

Institut für Mikrobiologie und Biotechnologie, Rheinische Friedrich-Wilhelms-Universität, Meckenheimer Allee 168, D-5300 Bonn, Germany

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Outer membrane (OM), cytoplasmic membrane (CM) and intracytoplasmic membranes (ICM) from the halophilic phototrophic purple sulphur bacterium *Ectothiorhodospira mobilis* 9903 were purified and characterized. The three membrane fractions were significantly different in regard to protein profiles on SDS-PAGE, and to the composition of amino acids, fatty acids and lipids. The presence of lipoproteins, the occurrence of lyso-phosphatidylethanolamine and an increased content of saturated and short-chain fatty acids are characteristic properties of the OM. CM and ICM fractions are different on the basis of buoyant density, of protein profiles and amino acid composition, and due to the presence of succinate dehydrogenase activity in CM. In addition, CM and ICM showed significant differences in pigment content and absorption spectra.

Introduction

Successful separation of cellular membrane fractions is essential for all investigations aiming at understanding the processes of membrane differentiation. For this reason, and in order to understand the differentiation of ICM structures, numerous attempts have been made to purify the ICM of phototrophic purple bacteria and eventually also to separate it from the CM (Niederman, 1974; Niederman & Gibson, 1978; Garcia & Drews, 1980; Drews & Oelze, 1981; Kaufmann *et al.*, 1982).

A number of investigations have dealt with the purification of 'chromatophores' of phototrophically grown cells of *Rhodobacter capsulatus* (Garcia & Drews, 1980; Kaufmann *et al.*, 1982), *Rhodobacter sphaeroides* (Niederman, 1974), *Rhodospirillum rubrum* (Collins & Niederman, 1976) and *Chromatium vinosum* (Hurlbert *et al.*, 1974). In most of these cases, OM or cell envelope fractions were obtained that were contaminated with ICM. Similarly, CM fractions were obtained that were contaminated with OM and with significant amounts of ICM. Much effort has been made to purify membrane fractions from *Rb. sphaeroides*. Niederman *et al.* (1979)

were the first to obtain an upper pigmented fraction in addition to the usual chromatophore fraction from phototrophically grown *Rb. sphaeroides*. These authors interpreted the presence of part of the photosynthetic apparatus in the presumed CM fraction as initiation sites of ICM formation. Membrane vesicles from phototrophically grown *Rb. sphaeroides* cells have also been isolated on a sucrose density gradient after prior removal of OM and osmotic shock of spheroplasts (Picorel *et al.*, 1990), but purification of OM and ICM was not attempted in this case. The ICM was found as a heavy fraction at 40% (w/v) sucrose. Comparable methods for the isolation of chromatophores or spheroplast-derived vesicles from the same bacterium were also used by Takemoto & Bachmann (1979) and by Hellingwerf *et al.* (1975).

In order to avoid cross-contamination with ICM, CM fractions have been isolated from chemotrophically grown purple bacteria (*R. rubrum* and *Rb. sphaeroides*; Oelze *et al.*, 1975; Guillotin & Reiss-Husson, 1975; Collins & Niederman, 1976). As an example, two different membrane fractions were isolated from aerobically grown *R. rubrum* cells (Oelze *et al.*, 1975; Collins & Niederman, 1976). The CM was found at the top of the sucrose density gradient at about 30% (w/v), the OM fraction at about 50% (w/v) sucrose. The lack of ICM under these growth conditions facilitates the purification of CM. It is not possible, however, to obtain information on differentiation processes under photo-

* Author for correspondence. Tel. 228 737719; fax 228 737576.

Abbreviations: OM outer membrane; CM cytoplasmic membrane; ICM intracytoplasmic membrane; BChl bacteriochlorophyll; LH I antenna complex I; LH II antenna complex II; RC reaction centre; SDH, succinate dehydrogenase.

trophic growth conditions from analysis of membranes from chemotrophically grown cells.

In the present communication, we report on a procedure to obtain different membrane fractions of *Ectothiorhodospira mobilis* 9903. Highly pure fractions of OM, CM and ICM from phototrophically grown cells were characterized and structural differences in the photosynthetic apparatus in CM and ICM fractions are indicated.

Methods

Strains and culture conditions. *Ectothiorhodospira mobilis* strain BN 9903 is an isolate from alkaline soda lakes of the Wadi Natrun in Egypt (Imhoff *et al.*, 1979, 1991). Cells were grown in 50 ml and 1 litre screw-cap bottles on the medium described by Imhoff (1988) containing 5% (w/v) total salts. Cells were incubated at 42 °C with an illumination of 20000–25000 lux.

Preparation of cell fractions. Cells were harvested in the late-exponential growth phase by centrifugation at 11000 g. The collected cell pellets were washed in 50 mM-Tris/HCl pH 9 containing 5% (w/v) NaCl and resuspended in the same buffer plus 30% (w/v) sucrose in one-tenth of the culture volume. Mureinoplasts were collected by renewed centrifugation. The supernatant was dialysed and recentrifuged at 200000 g to harvest the OM. The supernatant contained the soluble periplasmic proteins.

Mureinoplasts were resuspended and incubated for 3 h in Tris/HCl pH 9 with 0.5 mg lysozyme ml⁻¹. This procedure yielded protoplasts, which were washed in Tris/HCl pH 9, incubated for 30 min in 6 M-glycerol, 5 µg DNAase ml⁻¹, 1 µg RNAase ml⁻¹, and broken by osmotic shock by very rapidly adding 10 vols distilled water. The red supernatant from a low-speed centrifugation was further separated on a sucrose density gradient (20–60%, w/v) into two pigmented bands.

Protein determination. Protein was determined by the Lowry method. Pigments were extracted with ice-cold acetone/methanol (7:2, v/v). The pigment-free protein was collected by centrifugation, dried and used for protein assay. Bovine serum albumin served as a standard.

Analysis of pigments. Absorption spectra were measured with a Perkin-Elmer Lambda 2 double beam spectrophotometer using 1 cm quartz cuvettes. Membrane fractions were suspended in 50 mM-Tris/HCl pH 9 containing 60% (w/v) sucrose.

For the determination of bacteriochlorophyll *a* (Bchl *a*), 0.05 ml 1 M-Tris/HCl pH 8 was added to a 0.5 ml sample. This mixture was extracted with 4.5 ml acetone/methanol (7:2, v/v) for 30 min in the dark at 4 °C. The sample was centrifuged and the supernatant made up to 5 ml. Concentrations of Bchl *a* were calculated from the absorbance at 775 nm in acetone/methanol extracts by using $\epsilon = 82.3 \text{ M}^{-1} \text{ cm}^{-1}$ (Clayton, 1963).

For characterization of carotenoids, membrane fractions were extracted with acetone/methanol (7/2, v/v). Insoluble material was removed by centrifugation. This procedure was repeated until the supernatant was colourless. The total volume was adjusted to 0.5 ml. Pigments were separated by thin-layer chromatography on Sil G-50 plates (Macherey and Nagel, Düren, FRG). To prevent oxidation of the carotenoids during chromatography, plates were pretreated with sodium ascorbate and Livio plant oil. Samples and plates were always kept in the dark. Light petroleum (b.p. 60–80 °C)/propan-2-ol/water (100:12:0.25, by vol.) was used as solvent system. Reference strains with known carotenoids were used for identification of the pigment bands.

Amino acid analysis. Samples containing about 1 mg protein were dried and redissolved in 0.5 ml 6 M HCl containing 0.05% dithio-

erythritol. Proteins were hydrolysed in sealed tubes at 115 °C for 18 h under vacuum conditions. After hydrolysis the tubes were opened, dried in vacuum over KOH, and the contents redissolved in distilled water in order to remove the HCl completely. This procedure was repeated three times. The dried samples were finally dissolved in 3 ml sodium borate buffer (0.4 M, pH 9.5). Amino acids were analysed after derivatization with *o*-phthalaldehyde (OPA), as described by Sahl *et al.* (1985). Borate buffer (10 ml) was added to a 10 µl sample and 20 µl of this diluted solution was derivatized with 80 µl of OPA reagent. This reagent was kept as a stock solution of 56 mg OPA in 1 ml methanol and 10 µl 2-mercaptoethanol. For use, a tenfold dilution in borate buffer was prepared each day. After derivatization for 1 min, a 20 µl portion was injected onto a reversed phase C-18 column (Hypersil-ODS-5). Separation was achieved by using a gradient of 20–100% methanol containing 3% (v/v) tetrahydrofuran and 50 mM-sodium phosphate buffer pH 6.5. The OPA derivatives of the amino acids were detected with a Shimadzu fluorospectrophotometer equipped with a flow-through cell. Excitation and emission wavelengths were 340 nm and 450 nm, respectively. For quantitative evaluation, the peak areas were calibrated daily with a standard mixture containing 100 pmol of each amino acid. With this method, 15 different amino acids were detected.

Determination of phospholipid content. Phospholipids were extracted by the method of Bligh & Dyer (1959). The total phosphate content was determined by the method of Chen *et al.* (1956). Dried KH₂PO₄ was used as a standard. The total phospholipid content was calculated by assuming that 1 nmol phosphate corresponds to 84.7 ng phospholipid, and that the average molecular weight of the phospholipids is 800 (Bligh & Dyer, 1959).

Succinate dehydrogenase (EC 1.3.99.1) assay. The method of King (1963) and King & Drews (1973) was used. The reduction of 2,6-dichlorophenolindophenol at 600 nm mediated by phenazine methosulphate was measured. The reaction mixture contained 6 mM-sodium succinate, 4 mM-sodium azide, 0.04 mM-phosphate buffer pH 7.5, 0.06 mM-2,6-dichlorophenolindophenol and 0.4 mM phenazine methosulphate. The absorbance change was measured over a period of 15 min.

SDS-PAGE. Polyacrylamide gels were prepared according to the method of Laemmli (1970), with acrylamide concentrations of 10% and 15% (w/v).

Other methods. Fatty acids and lipids were analysed by procedures described previously (Imhoff, 1991; Imhoff & Thiemann, 1991). For staining proteolipids and lipoproteins in SDS-PAGE we used the method of Baumgardner *et al.* (1980). DNA and RNA determinations were made with orcin and diphenylamine as reagents according to Holtzhauer (1989). Antenna polypeptides of the membranes were isolated according to Brunisholz *et al.* (1984).

Results

Purification of OM, CM and ICM

In order to avoid cross-contamination between OM and other membrane fractions, we removed the OM of *Ectothiorhodospira mobilis* by treatment with 30% (w/v) sucrose prior to cell breakage. After separation from the mureinoplasts, high-speed centrifugation yielded an OM fraction that was free of photosynthetic pigments. The OM had a yellowish translucent, gel-like consistency, similar to an OM fraction from *Rb. sphaeroides* (Evers *et al.*, 1984). The mixture of CM and ICM, obtained after

lysozyme treatment, glycerol incubation and osmotic shock of the protoplasts, was separated on a 20–60% sucrose gradient. After successful osmotic shock, we obtained two coloured bands in the sucrose density gradients. The CM fraction was found at 30% sucrose (1.12 g cm^{-3}) and the ICM fraction at about 50% sucrose (1.23 g cm^{-3}).

The high purity of the membrane fractions obtained is demonstrated by the following observations. (i) All three membrane fractions were free of contamination by ribosomes and nucleic acids, as is indicated by absorption ratios (A_{260}/A_{280}) that were always less than 1.0. Also, chemical analyses with orcin and diphenylamine (Holtzhauser, 1989) did not give any indication of contamination of these fractions by DNA and RNA. The DNA/RNA content was always lower than $2 \mu\text{g (mg protein)}^{-1}$. (ii) Succinate dehydrogenase activity (SDH) was nearly exclusively found in the CM fraction. It was completely absent in the OM fraction. Specific activity of SDH was $2.2 \pm 0.6 \text{ nmol min}^{-1} \text{ (mg protein)}^{-1}$ in the CM fraction and $0.028 \pm 0.009 \text{ nmol min}^{-1} \text{ (mg protein)}^{-1}$ in the ICM fraction. (iii) Two bands, staining with Solvent Black 3, were detected after SDS-PAGE of OM fraction proteins. This reagent is used for specific detection of proteolipids and lipoproteins in SDS-polyacrylamide gels (Baumgardner *et al.*, 1980). The other two membrane fractions did not contain proteins staining with Solvent Black 3. (iv) Electron microscopy showed that the CM and ICM fractions were visually quite different (data not shown). Membranes of the ICM fraction had a distinct disc-shape, while those of the CM were irregular in form and size and often appeared as small vesicles. The former had a higher electron density than the latter fraction.

Absorption spectra and pigments

Remarkable differences were found in the absorption spectra (Fig. 1) and the pigment content of OM, CM and ICM fractions. Absorption maxima indicative of the presence of carotenoids, Bchl *a* and various forms of Bchl–protein complexes were absent from the OM fraction. Such absorption maxima were most pronounced in the ICM fraction. Absorption spectra of this fraction showed maxima at 800 nm and 850 nm characteristic of antenna complexes II (LH II) and of antenna complexes I (LH I) at 890 nm. Those of pure CM fractions indicated the presence only of the reaction centre (RC) and LH I complexes with maxima at 800 nm and 890 nm (Fig. 1).

Although both CM and ICM contained photosynthetic pigments, they showed significant differences in their qualitative and quantitative pigment content. At identical protein concentrations, the CM had a pink and the ICM a deep red colour. The amount of Bchl *a* in

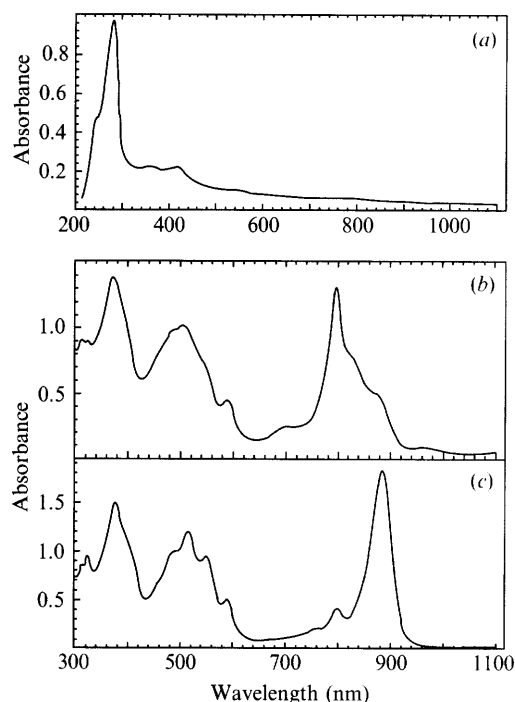


Fig. 1. Absorption spectra of purified OM (a), ICM (b) and CM (c) from *E. mobilis* 9903 grown at 5% total salts.

Table 1. *Phospholipid and BChl a content of OM, CM and ICM from E. mobilis* 9903

Fraction	BChl <i>a</i> concentration [$\mu\text{g (mg protein)}^{-1}$]	Phospholipid content* [$\text{mg (mg protein)}^{-1}$]
OM	Not detected	2.56
CM	7.76	0.85
ICM	91.79	0.71

* On the basis of the phosphate content of solvent-soluble lipids, calculated by the method of Chen *et al.* (1956).

the ICM was $92 \mu\text{g (mg protein)}^{-1}$ compared to $8 \mu\text{g (mg protein)}^{-1}$ in the CM (Table 1). Differences in pigment composition were also demonstrated by separating the carotenoids on TLC plates (data not shown). Spirilloxanthin was the major component in the ICM, besides lower contents of lycopene, rhodopin and rhodovibrin. In the CM fraction only spirilloxanthin was found, even in highly concentrated pigment fractions.

Protein profiles

Protein profiles of the three membrane fractions showed an individual and characteristic pattern (Fig. 2). The OM had four main polypeptide bands with M_r values of about 8000, 18000, 19000 and 26000. Two of these protein bands, with M_r values of about 8000 and 26000, also stained with Solvent Black 3. The ICM had five clearly dominating major polypeptide bands. These

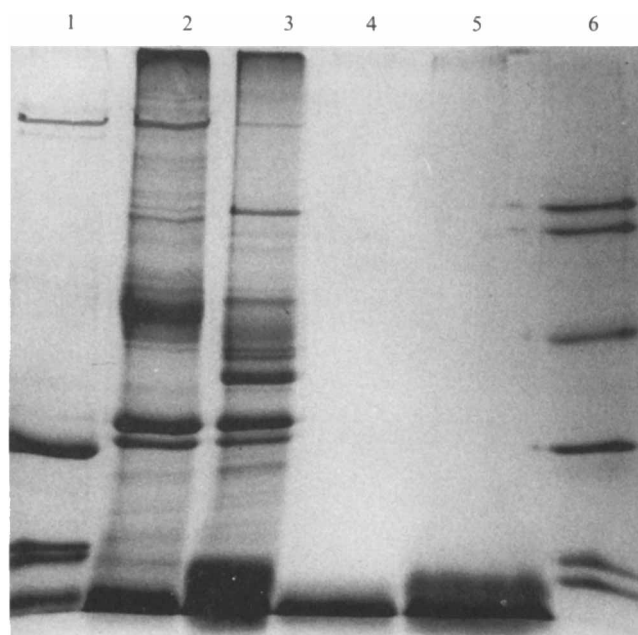


Fig. 2. SDS-PAGE (polyacrylamide concn 15% w/v) of OM (lane 1), CM (2) and ICM (3) fractions of *E. mobilis* 9903 grown on 5% total salts. Antenna polypeptides of CM (lane 4) and of ICM (5) are also shown. Lane 6, standard proteins: cytochrome *c*, M_r 12300; myoglobin, 17200; carbonic anhydrase, 30000; ovalbumin, 43000; albumin, 66250; ovotransferrin, 76–78000.

bands were present in solubilized and purified RC/LH I and RC/LH I/LH II complexes from these membrane fractions (unpublished results) and therefore can be regarded as constituents of the photosynthetic apparatus. Bands of M_r 7000 and 12000 were characteristic of LH I and LH II respectively. Bands of M_r 31000, 33000 and 37000 were constituents of the RCs of the photosynthetic apparatus. Polypeptides with M_r values greater than 70000 were not seen in the ICM fraction; they were characteristic, however, for the CM fraction, which contained a number of such polypeptides. Four polypeptides with M_r values of about 7000, 31000, 33000 and a broad band at about 47000 were quantitatively dominant in the CM. The last band was typical for this fraction. The antenna polypeptides of LH II of M_r 12000 and the band of M_r 37000, both major bands in the ICM, were absent in pure CM fractions. The isolation of antenna polypeptides from purified CM and ICM according to Brunisholz *et al.* (1984) supported differences in the composition of antenna complexes in those two membrane fractions (Fig. 2).

Amino acid composition

As with the protein composition, the amino acid composition of the three membrane fractions also revealed characteristic differences (Table 2). Asp, Ala

Table 2. Amino acid composition of proteins from OM, CM, and ICM of *E. mobilis* 9903

Values are percentages of the total amount of amino acids.

Amino acid	OM	CM	ICM
Asp	15.9	10.9	12.5
Glu	13.6	14.4	16.5
Ser	11.3	7.9	7.6
His	2.6	1.5	2.3
Thr	5.8	7.2	3.6
Gly	0.9	4.1	3.4
Arg	5.9	5.3	5.8
Ala	10.5	10.9	12.6
Tyr	4.5	13.5	4.8
Met	2.8	3.9	4.3
Val	9.1	4.6	4.8
Phe	3.2	6.5	5.9
Ile	2.8	4.8	4.1
Leu	5.2	1.5	7.6
Lys	5.9	3.0	4.4

and Glu were dominant in all membrane fractions. Characteristic was the high amount of Tyr (13.5%) in the CM and the high content of Glu and the low content of Thr in the ICM. Compared to the other membrane fractions, the OM had a higher content of Ser and Val, but a lower content of Gly and Ile.

A general property of proteins is determined by the polarity of their amino acids. This property may be expressed as the average hydrophobicity (Bigelow, 1967) or as the content of polar, intermediate and non-polar amino acids (Vanderkoi & Capaldi, 1972). Application of these parameters to the bulk proteins of our membrane fractions revealed significant differences. The average hydrophobicity of OM was 365 kJ per residue, of CM 445 kJ per residue and of ICM 397 kJ per residue. The content of polar amino acids amounted to 41.3% in OM, 33.6% in CM and 39.2% in ICM. Also different were the values of intermediate amino acids (OM 25.1%, CM 34.2%, ICM 21.5%) and non-polar amino acids (OM 33.6%, CM 32.2%, ICM 39.2%). Quite remarkable differences were also found between CM and ICM, not only in the relative content of individual amino acids, but also regarding the average hydrophobicity and proportions of polar and non-polar amino acids.

Lipids and fatty acids

No significant qualitative or quantitative differences in the polar lipid composition of CM and ICM were found in two-dimensional thin-layer chromatography separations (data not shown). The OM, however, had a higher content of cardiolipin and a lower one of phosphatidic acid. Lyso-phosphatidylethanolamine was detectable in the OM only (data not shown).

Table 3. Fatty acid composition of membrane fractions from *E. mobilis* 9903

Values are percentages of the total amount of fatty acids. ND, Not detected.

Fatty acid	OM	CM	ICM
C 12:0	0.3	ND	ND
C 14:0	2.4	0.2	0.6
C 14:1	0.4	ND	0.9
C 16:0	27.1	23.8	23.2
C 16:1	2.9	3.2	1.7
C 18:0	14.2	11.6	10.9
C 18:1	45.2	56.8	57.5
C 19 cyc	2.6	1.9	2.9
C 20:0	1.5	0.4	0.4
Sum of C 16	30.0	27.0	24.9
Sum of C 18	59.4	68.4	68.4
Sum of sat.	45.5	36.0	35.1
Sum of unsat.*	51.1	61.9	63.0

* 19 cyc included.

The content of fatty acids in solvent-extractable lipids from OM, CM and ICM is shown in Table 3. Although all membrane fractions had C 18:1 as the dominant fatty acid, its content was significantly lower in the OM (45.2%) compared to the two other membrane fractions. The C 18:1 content of the CM was 56.8% and that of the ICM 57.5%. A significantly higher content of short-chain and saturated fatty acids was characteristic for the OM. Major differences between CM and ICM are represented by the higher content of C 16 and the lower one of C 14 fatty acids in the CM.

Discussion

Numerous reports deal with the purification of ICMs of different phototrophic purple bacteria. In almost all investigations, methods for membrane separations involved harsh treatments such as French pressure cell passage or ultrasonication to disrupt cells and membranes. Various numbers of membrane fractions were obtained, which were classified as chromatophores, CM, OM or cell envelopes.

Five membrane fractions, for example, were collected from a sucrose gradient of *C. vinosum* cells disrupted by sonication (Hurlbert *et al.*, 1974). The uppermost three fractions, called 'light fractions', were deep red in colour. There was no decisive difference in the composition of these three membrane fractions, which all contained RCs and antenna complexes, a typical property of chromatophores. Fractions four and five, the 'heavy fractions', were light pink and turbid. They were regarded as mixtures of OM and CM. After French pressure cell disruption and sucrose gradient centri-

fugation, five membrane bands were also obtained from *Rb. capsulatus* (Kaufmann *et al.*, 1982). In this case the heavy fraction was characterized as chromatophores. All membrane fractions contained RC and antenna complex I polypeptides, whereas polypeptides of the antenna complex II were dominant in the heavy membrane fraction. Only three membrane fractions were found in another investigation with *Rb. capsulatus* (Garcia & Drews, 1980). The heavy fraction was characterized as the chromatophore fraction, although a clean separation of OM, CM and ICM was not obtained. SDH activity in the light fraction banding at 28–36% sucrose was higher than in the other membrane fractions, although activity was present in all fractions. Pure CM of *Rb. capsulatus* obtained from chemotrophically grown cells represented a light fraction (1.139 g cm⁻³) on sucrose gradients (Flammann & Weckesser, 1984). Fractions of CM from phototrophically grown cells have not been successfully purified and characterized. An ICM fraction from *E. mobilis* also was obtained by the sequential treatment of the cells with lysozyme, osmotic shock and differential centrifugation (Oyewole & Holt, 1976). Attempts to purify OM and CM and to analyse differences in the membrane fractions were not made.

Because all attempts to obtain fractions of CM and ICM after cell breakage by sonication or French press yielded several bands in sucrose density gradients and resulted in membrane fractions that showed significant cross-contamination of their protein bands, we broke cells by osmotic shock and developed the presented method for preparation of membrane fractions. Several lines of evidence indicate that our membrane fractions of OM, CM and ICM are highly pure.

(i) SDH activity is specifically associated with the CM of *E. mobilis* 9903. The activity in the ICM fraction was only about 1% of that in the CM fraction. The specific activity of SDH [2.2 nmol min⁻¹ (mg protein)⁻¹] in the CM fraction is comparable to those in the CM fractions of *Chloroflexus aurantiacus* (Feick *et al.*, 1982; 1.4 nmol min⁻¹ mg⁻¹) and of *Rb. sphaeroides* (Guillotin & Reiss-Husson, 1975; 4.7 nmol min⁻¹ mg⁻¹). Compared to values for *R. rubrum* (Oelze *et al.*, 1975; 39 µmol min⁻¹ mg⁻¹) and *Rb. capsulatus* (Flammann & Weckesser, 1984; 0.3 µmol min⁻¹ mg⁻¹), however, this activity is very low.

(ii) Each membrane fraction has a typical protein profile in SDS-PAGE. Major bands of OM were not detectable in CM and ICM. In addition, characteristic major protein bands of CM and ICM could not be detected in the other membrane fractions. This indicates lack of cross-contamination of the three membrane fractions. The main protein bands of ICM were those of the antenna complexes, with *M_r* values of about 7000 and 12000, and of the RC at 31000, 33000 and 37000.

These values compare to M_r values of 24800, 31300, 33000 and 36600 for the L, M and H RC subunits and a cytochrome of an unidentified *Ectothiorhodospira* species (Lefebvre *et al.*, 1989), and to M_r values of 26500, 34000 and 35000 for RC subunits from *E. mobilis*, *E. shaposhnikovii* and *E. halophila* (Leguijt & Hellingwerf, 1991).

(iii) The presence of lyso-phosphatidylethanolamine and bands staining with Solvent Black 3 in SDS-polyacrylamide gels is characteristic for the OM fraction. Lack of detectable quantities of these compounds in CM and ICM proves that the latter two are free of contamination with OM. Lyso-phosphatidylethanolamine was also found as a characteristic compound of the OM fraction of *R. rubrum* (Oelze *et al.*, 1975).

(iv) The absence of pigments in the OM demonstrates absence of contamination by CM and in particular ICM in this membrane fraction.

(v) CM and ICM have different absorption spectra and differences in pigment-protein complex composition. LH II complexes were found only in the ICM fraction. R. A. Niederman and co-workers were the first to obtain an upper pigmented membrane fraction different from the usual light fractions of *Rb. sphaeroides*. Although these authors used a French pressure cell to break the cells and did not remove the OM prior to breaking the cells, they were able to demonstrate that the pigment complexes of their upper band moved into ICM during further membrane development (Inamine *et al.*, 1984; Reilly & Niederman, 1986). They called the locations of the CM containing these pigment-protein complexes initiation sites of ICM development. The presence of such sites in the CM of *E. mobilis* could explain the presence of pigment-protein complexes in our CM fraction. LH II was found only in the ICM fraction and apparently is incorporated only in the developed or developing ICM. If we assume that LH II complexes are specifically inserted into the ICM only and that RC/LH I complexes are additionally located in the CM at so-called initiation sites of ICM invagination, then lack of LH II in CM fractions demonstrates lack of contamination by ICM and high purity of this membrane fraction. During osmotic shock, the presumed initiation sites remain attached to the CM.

ICM fractions of *E. mobilis* 9903 (this study) and *E. mobilis* 8113 (Oyewole & Holt, 1976) showed significant differences in their composition. Other membrane fractions were not characterized by Oyewole & Holt (1976).

Although the Bchl *a* content in the ICM fractions of *E. mobilis* 9903 and *E. mobilis* 8113 is nearly identical [92 and 85 µg (mg protein)⁻¹, respectively], differences between our results on ICM composition and the results of Oyewole & Holt (1976) are to be expected, because different strains were used in the two studies. Observed

differences also may be due to differences in growth conditions and growth phase of the cells investigated. We used cells from the exponential growth phase and grown at 42 °C compared to cells of *E. mobilis* 8113 from stationary growth phase and grown at 29 °C. The different growth conditions may be one important reason for differences in fatty acid compositions. It is known that fatty acid composition is strongly dependent on growth temperature and salinity, and that the proportion of 19 cyc increases towards the stationary phase at the expense of 18:1 (Imhoff & Thiemann, 1991). This explains the considerably higher content of 19 cyc (25.5%) in *E. mobilis* 8113 compared to our results (2.9%). The fatty acid composition of the isolated ICM fraction from *E. mobilis* 9903 is quite similar to that of whole membrane fractions of this strain grown at the same temperature (Imhoff & Thiemann, 1991). This can be expected, as the ICM represents the major part of the whole membrane fraction of *E. mobilis*.

Differences in the fatty acid composition between CM and OM of *E. mobilis* 9903 were similar to those found in other purple bacteria. A higher content of short-chain and saturated fatty acids and a reduced content of C 18:1 was also found in the OM fraction of *R. rubrum* (Oelze *et al.*, 1975). Similar trends were also found in *Rb. capsulatus* (Flammann & Weckesser, 1984) and *C. vinosum* (Hurlbert *et al.*, 1974). It is concluded that this indicates a general phenomenon among Gram-negative bacteria.

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